European Journal of Applied Sciences 3 (3): 71-74, 2011
ISSN 2079-2077

In-Vitro Antimicrobial Activity of *Coccinia grandis* on Ulcer Producing *Helicobacter pylori*

P. Poovendran, V. Kalaigandhi and E. Poogunran

Department of Microbiology, Dr. G.R. Damodaran College of Science,
Coimbatore - 641 014, Tamil Nadu, India

Abstract: Ulcer producing organisms have evolved numerous defense mechanisms against antimicrobial agents, hence resistance to old and newly available drugs are increasing at an unprecedented level. The events of antibiotic resistance have lead for screening of several medicinal plants for their potential antimicrobial activity. The aim of this study is to evaluate the antimicrobial efficacy of *Coccinia grandis* against ulcer producing *Helicobacter pylori* (ULHP). *C. grandis* is a widespread medicinal plant traditionally used in India to treat infectious diseases. Aqueous, acetone and ethanol extracts of leaves of *C. grandis* were tested for antimicrobial activity *in vitro* by the agar well diffusion method. Ethanol extract of leaves exhibited antimicrobial activity against ulcer producing strains ULHP 2 and 4, whereas the aqueous and acetone extracts showed antibacterial activity only against ULHP 3. These antimicrobial properties seem to be related to the presence of tannin, alkaloids and tri-terpenoids in *C. grandis*. It can be concluded that *C. grandis* can be used to discover natural products that may serve as lead for the development of new pharmaceuticals, addressing the major therapeutic needs especially for ulcer producing pathogenic strains.

Key words: *Helicobacter pylori* · *C. grandis* · Antimicrobial activity

INTRODUCTION

Helicobacter pylori are a gram negative helical rod that colonizes the human gastric mucous layer [1-3]. It chronically infects the gastric mucosa causing gastritis in more than 50% of the world's population. The infection can lead to the development of peptic ulcer [4] and gastric mucosa-associated lymphoid tissue lymphoma [5]. Infection with the organism has also been linked with an increased risk of gastric cancer in humans [6, 7].

The development of safe anti-*Helicobacter pylori* compounds is desirable due to the problem of antibiotic resistant strains that have emerged [8]. Currently, new triple therapies consisting of two antibiotics and a proton pump inhibitor demonstrate considerable eradication rates. However, with the organism rapidly acquiring resistance to some of these antibiotics coupled with the fact that some of these drugs occasionally cause side effects, as well as their high cost for treatment, there is need to search for alternative therapies [9]. In the recent past, the rapid development of multidrug resistant bacterial strains of clinically important pathogens fetches the interest of scientists to develop newer broad spectrum antimicrobial agents [10].

The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in different traditional literatures [11, 12]. Studies have documented that some medicinal plant extracts have antibacterial activities,

H. pylori is inclusive [13-16]. *Coccinia grandis* (L.) belongs to family *Cucurbitaceae*, commonly known as Kundru in Hindi and Ivy Gourd in English, is a vegetable grown wildly throughout India [17]. Every part of this plant is valuable in medicine for ring worm, psoriasis, small pox, scabies [18] and other itchy skin eruptions and ulcers [19]. The plant can also be used to treat cough [20]. The leaves of the plant possess antimicrobial, anti-diabetic, antipyretic, anti-inflammatory, antispasmodic, cathartic and expectorant activities [21, 22]. The leaves of this plant contain tri-terpenoids, alkaloids and tannins [23].

The aim of this study was to substantiate the antimicrobial sensitivity of different extracts of *C. grandis* leaves against clinical isolates of ulcer producing *Helicobacter pylori* strains to lengthen the queue of antimicrobial herbs.

Corresponding Author: V. Kalaigandhi, Department of Microbiology, Dr. G.R. Damodaran College of Science, Civil Aerodrome Post, Avanashi Road, Coimbatore- 641 014, India.
Mob: 099945 83133, E-mail: lvkalai2001@yahoo.com.

MATERIALS AND METHODS

Collection of Plant Materials: Leaves of *C. grandis* were collected from villages in and around Coimbatore district, South India. Plant leaves were dried under the shadow. The dried leaves were fine powdered and stored in polythene bags at room temperature (30°C) until use.

Extract Preparations

Aqueous Extract: To obtain the aqueous extracts, about 10 grams of the dried and finely powdered leaves of *C. grandis* were homogenized using 100 ml of water. They were added to Soxhlet apparatus and the boiling point of water was set up at 100°C. The water evaporates continuously and was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution loses the color (24).

Acetone Extract: Ten grams of the dried and finely powdered leaves of *C. grandis* were homogenized using 100 ml of acetone. They were added to Soxhlet apparatus and the boiling point of acetone was set up at 56.6°C. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent loses its color. The extract was then transferred to a sterile Petri dish and kept for evaporation of acetone at room temperature. Residues of extracts were collected and stored in the refrigerator.

Ethanol Extract: Ten grams of the dried and finely powdered leaves of *C. grandis* were homogenized using 100 ml of 70% ethanol. They were added to Soxhlet apparatus and the boiling point of ethanol was set up at 78°C. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent loses its color. The extract was then transferred to a sterile Petri dish and kept for evaporation of ethanol at room temperature. Residues of extracts were collected and stored in the refrigerator.

Antibacterial Activity of Plant Extracts: Antibacterial activity of the aqueous, acetone and ethanol extracts of leaves of *C. grandis* was tested using the agar well diffusion method (24). Four ulcer producing strains were employed for testing the antimicrobial activity of the aqueous, acetone and ethanol extracts of leaves of *C. grandis*; ULHP 1, ULHP 2, ULHP 3 and ULHP 4. The selection of the test organisms was based on the resistant pattern exhibited against the antibiotics used to treat ulcer caused by *H. pylori*. A loop full of culture of each test strain was inoculated into peptone broth and incubated for 2 to 6 hours at 37°C until it achieved the turbidity of 0.5 McFarland's standard. The test cultures were swabbed on nutrient agar plates, within 15 minutes after adjusting the turbidity of the inoculums suspension. Wells were made using the sterile well puncture. Different concentrations (200µg to 1000µg) of the sterile aqueous, acetone and ethanol extracts were added to each well. The plates were incubated at 37°C for 24 hours. The diameter of inhibition zones were measured in millimeter (mm) and the results were recorded.

RESULTS AND DISCUSSION

In vitro antibacterial activities of leaves of *C. grandis* against *Helicobacter pylori* are shown in Table 1. The aqueous and acetone extracts of (200-1000µl) *C. grandis* leaves showed no significant zone of inhibition against the tested strains except ULHP 3 with inhibition zone diameter about 22 and 26 mm, respectively achieved at the highest extracts concentration (1000 µg/ml).

It is clear from the Table 1 that the ethanol extract of *C. grandis* leaves have exhibited antimicrobial activity against ULHP 2 and ULHP 4 with maximum zones of inhibition of 22 and 24 mm, respectively, but failed to exhibit inhibitory action against ULHP 1 and ULHP 3.

The antimicrobial activities of various plants have been reported by many researchers [24, 25]. Umbreen *et al.* [26] reported the significant activity of methanol and ethyl acetate extracts of leaves and stem of *C. indica* against different bacteria providing a support to the fact that methanol is a better solvent for extraction and isolation of phytochemicals having antimicrobial activity. The present study revealed that the ethanol extract was found to be active against two ulcer producing strains namely ULHP 2 and ULHP 4 and resistant against ULHP 1 and ULHP. Dewanjee *et al.* [27] have reported that methanol extract of *C. grandis* leaves exhibited significant antimicrobial activity.

In this study, the water extract displayed lower antibacterial activity than acetone and ethanol extracts. This is in agreement with earlier studies which reported that use of organic solvents is always better for extraction of antibacterial compounds [28]. Furthermore, the effectiveness of the extracts are not due to one main constituent, but to the combined action of other chemical compounds involved in it [29]. Some of them include

Table 1: Antimicrobial activity of the different extracts of *C. grandis* leaves by well diffusion method against *Helicobacter pylori*

		Ulcer Producing *H. pylori*			
Plant Extract (1000µg/ml)	Solvent	ULHP 1	ULHP 2	ULHP3	ULHP 4
C. grandis	Aqueous	-	-	22	-
	Acetone	-	-	26	-
	Ethanol	-	22	-	24

ULHP= Ulcer Producing *Helicobacter pylori*, '-' Indicates no significant zone of inhibition

alkaloids, flavonoids, terpenoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds [30]. The results of this study showed that the *C. grandis* leaves have exhibited varied antimicrobial activities against the ulcer producing *H. pylori*. These findings support the claim of the traditional healers that *C. grandis* would be used against ulcer pathogens.

REFERENCES

1. Shimizu, T., T. Akamatsu, A. Sugiyama, H. Ota and T. Katsuyama, 1996. *Helicobacter pylori* and the surface mucous gel layer of the human stomach. Helicobacter, 1: 207-208.
2. Hayama, M., Y. Kawakami, Y. Kanelo, K. Sona and H. Ota, 2005. *Helicobacter pylori* infection increases cell kinetics in human gastric epithelial cells without adhering to proliferating cells. J. Cell Mol. Med., 9: 746-747.
3. Ndip, R.N., A.E. Malange -Tarkang, J.E.A. Ojongokpoko, H.N. Luma, A. Malongue, J.F.K. Akoachere, L.M. Ndip, M. MacMillan and L.T. Weaver, 2008. *Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: current status of antibiogram. Trop Med Inter Health., 10: 848-854.
4. Sontag, S.J., 1997. Guilty as charged: bugs and drugs in gastric ulcer. Amer. J. Gastroenterol., 92: 1255-1261.
5. Du, M.Q. and P.G. Isaccson, 2002. Gastric MALT lymphoma: from aetiology to treatment. Lancet Oncol., 3: 97-104.
6. Forman, D., P. Webb and J. Parsonnet, 1994. *Helicobacter pylori* and gastric cancer. Lancet., 343: 243-244.
7. Uemura, N., S. Okamoto, S. Yamamoto, N. Matsumura, S. Yamaguchi, M. Yamakido, K. Taniyama, N. Sasaki and R.J. Schlemper, 2001. *Helicobacter pylori* infection and the development of gastric cancer. New Engl J Med., 345: 784-789.
8. Yoshiyuki, K., O. Kozue, H. Masayoshi, O. Hiroyoshi, T. Masahiko, M. Kazuhiro, M. Tsunetomo and K. Kiyomi, 2006. *In vitro* bactericidal activities of Japanese rice-fluid against *H. pylori* strains. Int. J. Med. Sci., 3: 112-116.
9. O'Connor, H.J., C. Kanduru and A.S. Bhutta, 1995. Effects of *H. pylori*eradication on peptic ulcer healing. Postgrad Med. J., 71: 90-93.
10. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin Microbiol. Rev., 12: 564-582.
11. Isogai, H., E. Isogai and S. Hyashi, 2000. Antibacterial activities of catechin as phytochemical substances. Res Adv Antimicrob Agents Chemother., 1: 13-18.
12. Funtogawa, K., S. Hayashi and H. Shimomura, 2004. Antibacterial activity against *H. pylori*. Microbiol Immunol., 48: 251-261.
13. Ndip, R.N., A.E. Malange -Tarkang, S.M. Mbullah, H.N. Luma, A. Malongue, L.M. Ndip, C. Wirmum and S.M.N. Efange, 2007. *In vitro* anti-*Helicobacterpylori* activity of extracts of selected medicinal plants from North West Cameroon. J. Ethnopharmacol., 114: 452- 457.
14. Weisser, R., A.W. Asscher and J. Winpenny, 1966. In vitro reversal of antibiotic resistance by DTA. Nature, 219: 1365-1366.
15. Satish, S., K.A. Raveesha and G.R. Janardhana, 1999. Antibacterial activity of plant extracts of phytopathogenic *Xanthomonas campestris pathovars*. Letter Appl. Microbiol., 28: 145-147.
16. Jones, F.A., 1996. Herbs-useful plants, their role in history and today. European J. Gastroenterology and Hepatol., 8: 1227-1231.
17. Brahmachari, H.D. and K.T. Augusti, 1963. Orally effective hypoglycemic principles from *Coccinia indica*. J. Pharmacy and Pharmacol., 15: 411-412.
18. Perry, L.M., 1980. Medicinal Plants of East and South East Asia, Attributed properties and Uses, MIT Press, London.
19. Behl, P.N., R.B. Arora, G. Srivastava and B.N. Malhotia, 1993. Herbs useful in Dermatological therapy, CBS Publishers and Distributor, Delhi.

20. Sharma, H.K., L. Chhangte and A.K. Dolui, 2001. Traditional medicinal plants in Mizoram, India. Fitoterapia, 72: 146-161.
21. Asolkar, L.V., K.K. Kakkar and O.J. Chakre, 1992. Second supplement to Glossary of Indian Medicinal Plants with Active Principles Part-I (A-K). Council of Scientific and Industrial Research (PID), New Delhi, (Part-1), pp: 217-218.
22. Nadkarni, K.M. and A.K. Nadkarni, 1992. Indian Materia Medica 3rd Ed. Popular Prakashan Pvt. Ltd., Mumbai, 1: 300-302.
23. Rastogi, R.P. and B.N. Mehrotra, 1990. Campendium of Indian Medicinal Plants. New Delhi, 1: 115.
24. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Review., 22: 564-582.
25. Shariff, Z.U., 2001. Modern Herbal Therapy for common Ailments. Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom, Nature Pharmacy Series, 1: 9-88.
26. Umbreen Farrukh, Huma Shareef and Shaukat Mahmud, 2008. Antimicrobial activity of *Coccinia grandis*. Pakistan J. Biol., 40(3): 1259-1262.
27. Dewenjee, S., M. Kundu, A. Maiti, A. Majumdar and S.C. Mandal, 2007. In Vitro evalution of antimicrobial activity of crude extract from plants *Diospyros peregrine*, *Coccinia grandis* and *Swietenia macrophylla*. Tropical J. Pharmaceutical Res., 6(3): 773-778.
28. Varadarajan, M., V. Guruchandran, S.M. Nagarajan, E. Natarajan, R. Punitha, C. Geetha and S. Ramya, 2007. Screening for the evaluation of antibacterial activity of *Solanum nigrum* L. Asian J. Microbiol. Biotechnol. Sci., 9: 79-81.
29. Bai, D., 1990. Traditional Chinese material: A respect and prospect. Plant Medicine, 50: 502.
30. Rojas, A., L. Hernandez, R. Pereda-Mirands and R. Meta, 1992. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican Medicinal Plants. J. Ethano Pharmacol., 35: 275- 283.